



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 48/00, C12N 15/85	A1	(11) International Publication Number: WO 97/07826 (43) International Publication Date: 6 March 1997 (06.03.97)
(21) International Application Number: PCT/US96/13591 (22) International Filing Date: 26 August 1996 (26.08.96) (30) Priority Data: 60/002,911 29 August 1995 (29.08.95) US Not furnished 16 August 1996 (16.08.96) US (71) Applicants: CBR LABORATORIES, INC. [US/US]; 800 Huntington Avenue, Boston, MA 02115 (US). UNIVER- SITY OF SOUTH FLORIDA [US/US]; 4202 East Fowler Avenue, FAO 126, Tampa, FL 33620 (US). (72) Inventors: NICOLAU, Claude, Y.; 54 LaGrange Street, Chest- nut Hill, MA 02167 (US). HELLER, Richard; 4405 Vieux Carre Circle, Tampa, FL 33613 (US). ATKIN, Andrew; 6 Warner Street, Nashua, NH 03063 (US). GILBERT, Richard; Address not furnished (**). JAROSZESKI, Mark; Address not furnished (**). (74) Agent: SACKS, Stanley; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM). European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: IN VIVO ELECTROPORATION OF CELLS (57) Abstract A method of treating living mammals in vivo is carried out by introducing foreign nucleic acid by electroporation into cells in vivo to cause suppression or expression of at least one protein in vivo. The method can be carried out to alter genetic characteristics of cells in vivo.		

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IN VIVO ELECTROPORATION OF CELLS

Related Application

This application claims the benefit of U.S. Provisional Application No. 60/002,911, filed
5 August 29, 1995.

Field of the Invention

This invention relates to electroporation of living cells in vivo for intracellular delivery of
nucleic acids including genes of interest for the expression of desired protein or antisense
10 nucleotides for the suppression or expression of certain intracellular proteins.

Background of the Invention

Liposomes and positively charged polymers like poly-L-Lysine have been used during
the past years as in vivo delivery systems for gene therapy and cancer treatment, (Nicolau et al
15 Proc. Natl. Acad. Sci. USA 80, 1068-1072, 1983 Duzgunes N. and Felgner P.L., 1993, Methods
Enzymol., 221:303-306). However, these approaches have clearly demonstrated serious
disadvantages including low efficiency in vivo and relatively low efficacy of intracellular DNA
delivery. The nature of liposome delivery to cells in organs other than liver and lung make
liposomes a relatively inefficient delivery vehicle for gene therapy for various practical
20 applications including treatment of many solid tumors.

Electroporation has been shown to be the most efficient method for DNA delivery in
vitro (Neuman et al, 1982, EMBO J., 1:841-845). The mechanism of DNA transfer in this
method is based on formation of membrane pores followed by DNA electrophoresis through
these pores into the cells (Neuman E. et al., 1982, EMBO J., 1:841-845). The mechanism of
25 electroporation does not demonstrate many of the disadvantages of other gene transfer
approaches. Moreover, the procedure can be applied to any cell type without limitations.

Summary of the Invention

It is an object of this invention to provide a method for treating living cells as in normal
30 as well as cancer cells of organs or solid tumors of all kinds, in vivo, by introducing foreign
nucleic acid in vivo by electroporation to cause suppression or expression of at least one protein
in vivo.

It is a still further object of this invention to provide a method of treating living cells in vivo by introducing foreign nucleic acids using electroporation, into cells in vivo to act as gene therapy for a multitude of disorders.

Still another object of this invention is to provide treated cells in accordance with the treatment of the methods of this invention.

According to the invention, a method of treating living cells in vivo comprises introducing foreign nucleic acids by electroporation into cells in vivo to cause suppression or expression of at least one protein in vivo. The method can be carried out as a means of introducing genes into cells in vivo.

In a preferred embodiment, the nucleic acid introduced alters protein expression to make the cells sensitive to destruction when treated with a drug nontoxic to normal cells. This method is useful as a cancer treatment for man, animals and mammals.

It is a feature of this invention that known electroporation techniques can be used in vivo to alter living cells and thus affect and treat humans and animals. In many cases, unwanted tumors and genetic defects and diseases can be prevented and/or cured, or at least partially cured. The nucleic acids including DNA is passed into living cells in vivo through the cell membrane and is taken up in the cell DNA as known in the art.

Brief Description of the Drawings

The above and other objects, advantages and features of the present invention will be better understood from a reading of the following specification in conjunction with the accompanying drawings, in which:

Fig. 1 is a diagrammatic illustration showing a top plan view of electrode placement for electroporation in an exposed medial lobe of a rat liver;

Fig. 2 is a diagrammatic cross-sectional view through the liver of Fig. 1 diagrammatically showing the needles and injected DNA;

Fig. 3 is a summary of luciferase activity 48 hours after electroporation using the methods of this invention with pulses at varying field strength;

Fig. 4 shows expression of luciferase expression after electroporation;

Fig. 5 shows the dose response curve for luciferase expression plating the dose injected into a rat liver or control (no injection of DNA, just electroporation) against luciferase activity in AU arbitrary light units;

Fig. 6 is a flow cytometry assay of β -galactosidase expression after in vivo electroporation without the gene injected; and

Fig. 7 is the same as Fig. 6 with the gene injected at 25 mg/ml. Each histogram of Figs. 6 and 7 plots the log of fluorescent intensity (abscissae) versus number of cells population

5 (ordinate).

Detailed Description of the Preferred Embodiment

Generally, living cells can be treated in vivo by introducing foreign nucleic acid by electroporation into cells in vivo to cause suppression or expression of at least one protein in vivo. The method can be used to treat living cells by introducing foreign nucleic acid such as genes into cells for gene therapy to alter a genetic characteristic of the cells as, for example, to correct genetic problems and disorders as in replacement gene therapy and in cancer treatment.

The nucleic acids to be injected into cells in vivo, as the term is used herein, includes nucleic acids of any size, whole or partial genes, DNA, RNA and generally any nucleic acid sequence which are injected by electroporation. The nucleic acids can be foreign nucleic acids or can supplement nucleic acids already present in the cells.

As used herein, "cells" are meant to include living cells, organs, tissues and solid cancers. Thus, malignancies can be treated, particularly solid tumors, by the methods of this invention as will be described.

20 The cells are meant to include cells of man and animals including, but not limited to, all mammalian species.

In vivo treatment is treatment in or at the body while the cells are living as, for example, electroporation of organs through the skin of the body or after surgical procedures to expose organs. In some cases, electroporation can be carried out with the use of electroporation needle displays which are of extremely small size and which can be combined in cannula and endoscopic fiber optic instruments. Endoscopes are preferred instruments for reaching internal solid tumors since they can carry viewing optics as well as nucleic acid injection needles and electrodes allowing in vivo steps to be carried out internally without the need for surgery. It is preferred that needle penetration for injection of nucleic acids and application of an applied voltage, preferably follow a multi-needle design as, for example, shown in Figs. 1 and 2 where the needles are shown to penetrate a site in the exposed medial lobe of a rat liver, Fig. 2 shows a cross section thereof with DNA previously injected through hypodermic syringes/needles, which

also apply the voltage. Preferably two different electrode designs are used. In the first, there is a central electrode needle surrounded by eight additional needles. The eight electrode needles are equispaced around a 0.5 cm radius from the center needle. The conductive needles can be 0.3 millimeter diameter. This electrode is inserted so that the central needle enters the center of the area for the quantity of DNA to be injected as shown in Fig. 1. The second electrode utilizes six needles that can be equispaced around 1 cm diameter. The electrode can be inserted so that the site of the DNA injection is within a circular region encompassed by the needles. In all cases, the needles can protrude for example approximately 5 mm into the liver or other cells, organs, cancers or tissues which are to be treated.

Electroporation techniques for injection into cells in vitro are well-known in the art and such known techniques can be used, see Eur. J. Cancer, Vol. 27, No. 1, pp 68-72, 1991, Mir et al., Biochemical and Biophysical Research Communications, pp 938-943, Vol. 194, No. 2, 1993, Salford et al. and Cancer, Vol. 72, No. 12, 1993, pp 3694-3700, Beuhradeh et al., all of which articles are incorporated herein by reference.

It is preferred that the needle arrangement of the electrode be arranged to give the smallest overall diameter of the electrode needle array. Preferably, plural needle arrays are incorporated in endoscopic instruments of known types. The electrodes pass through the endoscopes. Thus, the needles can be used to treat remote areas in the body accessed through endoscopes as, for example, treating solid tumors of the pancreas, brain, gastrointestinal tract, liver, skin, kidneys, gynecological tumors and cancer of other organs of the body.

Electroporation in vivo for the local intracellular delivery of nucleic acid can be achieved by the local injection of any desired nucleic acids. Nucleic acids as used in this application include DNA, RNA and synthetic oligonucleotides. The nucleic acids can be injected into the tissue of interest followed by either a single or multiple rectangular pulse or exponential electric pulses with strength ranging from 200 V/cm to 1,500 V/cm and the time constant ranging from 1 microsecond to 100 millisecond. The nucleic acid is used in aqueous or other suspension, with the concentration ranging from 0.001 mg/ml to 10 mg/ml and is injected into the tissue of interest in a dose ranging from 0.001 to 10 mg/kilogram or more. The electric pulses must be essentially applied locally to the tissue injected with said nucleic acid of interest within period of time ranging from 5 seconds to 30 minutes after injection using needle-like electrodes from 1 mm to 5 mm in length and separated by 0.4 mm to 20 mm distance from each other. The multiple pulses with different parameters including electric field strength, time constant and

shape can be applied to the tissue injected with the said nucleic acid for an efficient electroporation of cellular membrane and intracellular delivery by means of transmembrane electrophoresis.

The preferred electroporation device in the present invention consists of a fiberoptic
5 endoscopic component combined with injection needles connected to a syringe for injection and needle-like electrodes connected to an electric pulse generator. The injection needle and needle-like electrodes are normally within the endoscope device and can be moved in the operating position and inserted into a tissue independently under the visual control of a fiberoptic component of the device.

10 Genes which can be transferred to tumor or cancer cells for therapeutic purposes can be any of the known genes for any known therapeutic purposes. Specific useful genes include:

herpes virus thymidine kinase. Wagner M.J. et al. Proc. Natl. Acad. Sci. USA, Vol. 78, pp 1441-1445, 1981,

interleukin-2 (IL-2), Taniguchi T et al., Nature, Vol. 302, pp 305-310, 1983 and Devos R.,
15 et al., Nucleic Acid Research, Vol. 11, pp 4307-4323, 1983,

HLA-B4403, Fleischhauer K et al., Tissue Antigens Vol. 37, pp 133-137, 1991,

Gene for Encoding Receptor for Low Density Liposuction, Wilson, J.M. et al., J. Biol. Chem., Vol. 267, pp 963-967, 1992.

Genes which can be injected in vivo for replacement therapy for genetic disorders include
20 those encoding for β -glucocerebrosidase (Gaucher disease) and α -25-hydroxylase (phenylketonuria).

The application of the described in vivo electroporation method can be demonstrated using a rat model. Rat livers can be electroporated in vivo with a plasmid carrying the luciferase gene site or the gene encoding for β -galactosidase as a reporter. The conditions described in the
25 first example below can be used to inject the luciferase gene or the β -galactosidase into a rat liver. The animals can be sacrificed at various times after electroporation and luciferase activity can be measured in a liver homogenate. The data shown in Figs. 3-5 indicate that luciferase expression can be observed for at least 21 days after an electroporation procedure, according to the first example below.

30 In a first specific example of this invention a protocol for gene transfer into rat liver organs is established. A 3 mg/kg dose of atropine is administered by subcutaneous injection into

the right flank of a male rat. Ten minutes later a 45 mg/kg dose of sodium pentobarbital is introduced by intraperitoneal injection.

The rat is then completely anesthetized and the liver is surgically exposed. This is preferably done by making a transfer incision starting from the mid-sagittal position,

5 approximately 1 cm caudal to the xiphoid process, extending 3 to 4 cm toward the dorsal surface of the rat. The medial lobe (both halves) of the liver is exposed by drawing it out of the incision without causing tissue damage. Nucleic acid or DNA is injected into the right central lobe of the exposed liver tissue. Typically 100 μ g of DNA in 100 μ l of sterile injectable saline are used. A time period of 1.5 minutes is allowed to elapse after injection in order to allow for diffusion of
10 the DNA. Electrodes are inserted into the liver tissue.

Two different electrode designs are used. The first is comprised of a central needle surrounded by eight additional needles. The eight needles are equispaced around a 0.5 cm radius from the center needle. This electrode is inserted so that the central needle enters the center of the injected quantity of DNA (see Figure 1). The second electrode utilizes 6 needles that are
15 equispaced around a 1 cm diameter. This electrode is inserted so that the site of nucleic and/or DNA injection is within the circular region encompassed by the needles. All needles on both electrode types protrude 5 mm. Pulses are administered to the tissue. Pulsing is slightly different for each of the two electrode types. For the 9 needle electrode, eight 99 μ second rectangular DC pulses with an electric field strength of 1000 V/cm and a duty cycle of 1 second
20 are delivered. The center needle functions as the anode and all eight needles positioned around the annulus of the electrode body function as the cathode. The distance between the center needle and any annular needle is 5 mm. The 6 needle electrode uses a rotating electric field. For this type of pulsing six 99 μ second rectangular DC pulses are delivered. The electric field strength is 1000 V/cm, and the duty cycle is 1 second. The area that was encompassed by the
25 electrode is marked using sutures. The electrode is removed, and the liver is placed back into its natural position. The incision is closed with surgical staples. At the desired time after treatment, the animal is euthanized by administering a 100 mg/kg dose of sodium pentobarbital by intraperitoneal injection. The treated tissue is then removed for analysis.

Results are obtained which demonstrate that electroporation can be efficiently used in
30 vivo. Pulses with the field strength up to 2 kV/cm were applied to the live tissues without affecting experimental animals but the optimal field strength for electroporation appears to be significantly lower. Fig. 3 summarizes the results of these experiments. Electric pulses with the

field strength between 1 kV/cm and 2 kV/cm allow an efficient transfection of liver cells and a high level of luciferase expression as measured at 48 hours after electroporation. The field strength above 2 kV/cm led to necrosis of the electroporated tissue, possibly due to the insufficient heat dissipation. The level of expression in electroporated cells is probably higher than the one we detected experimentally because all measurements were performed in the liver tissue homogenate from a broad area covering the electroporated region. Accurate location of the electroporated area is limited by technical factors.

Detection of luciferase expression at various times after in vivo electroporation demonstrates that the level of luciferase activity in tissue reaches the maximal value at 48 hours followed by decrease to 5-7% of the initial activity during the first week and remains stable for three weeks after electroporation (Fig. 4). These results may indicate that the long term-expression of reporter gene might be supported by stable transfectants because the transient expression achieved with other transfection methods decreases gradually during 2-3 weeks after transfection.

The efficiency of the reporter gene expression after in vivo electroporation strongly depends on the amount of plasmid DNA and optimal results were detected using 25 μ g of DNA while an increase in this amount leads to the reduction of luciferase activity (Fig. 5). These results differ from data regarding electroporation in vitro where DNA dose-response curve clearly reaches the saturation at concentrations above 100 μ g/ml. There is not presently an explanation of this difference.

One of the important advantages of electroporation in vitro is a high efficiency of intracellular DNA delivery. To assay the efficiency of the in vivo procedure, the liver tissue was transfected with the β Gal gene as described above in the first example. Hepatocytes were isolated from the dissected liver tissue using Collagenase treatment. The isolated cells were treated with chloroquine to block lysosome activity followed by incubation with the lipophilic β Gal substrate C₁₂FDG (Molecular Probes Eugene Oregon). The activity of the reporter gene was assayed using flowcytometry. Fig. 6 shows results of this experiment. Cells isolated from mock-electroporated liver tissue showed no β Gal activity, whereas the cell suspension prepared from β Gal-transfected liver contains about 30% of β Gal-positive cells. The real number of efficiently transfected cells in the electroporated area might be significantly higher due to the uncertainties in the accurate location of the transfected tissue.

In a further example, the treatment of melanoma is achieved by an injection of 0.1-0.5 ml of plasmid DNA carrying the herpesvirus gene of thymidine kinase under the control of the CMV promoter with a concentration in normal saline of about 1 mg/ml followed by DNA diffusion in the tumor tissue for about 2 min. The needle-like electrodes mounted according to Fig. 1 are inserted into an in vivo melanoma tumor and surrounding skin tissues of a male human patient. Eight rectangular electric pulses of 99 μ sec with electric field strength of 1000 V/cm are delivered to the tumor tissue with 1 second intervals for 15 min. Ganciclovir is administered to a patient at therapeutical doses of 5 mg per kilogram weight of the patient intravenously every twelve hours for 14 days. The nontoxic ganciclovir drug is converted into a toxic compound by thymidine kinase expressed in the electroporated tumor cells with cytotoxic effect on these treated cells and neighboring cells.

In another embodiment of the present invention, the treatment of pancreatic tumors of either acinar or endocrine phenotype is achieved by providing endoscopic electroporation. An endoscopic device comprising a fiber optic component, injection needle and electroporation needle-like electrodes is passed through the esophagus and the front end of the instrument is moved to the tumor site under the control of the fiber optic component of said device. The injection needle is moved forward from the device and inserted into the tissue under visual control using a fiber optic component of the device. An injection of 0.1-0.5 ml of plasmid DNA carrying the herpesvirus gene of thymidine kinase under the control of the CMV promoter with concentration of about 1 mg/ml is injected into the tumor. Injected DNA is allowed to diffuse in the tumor tissue for 5 min. Six circularly arranged needle-like electrodes mounted within the device are moved at working position from said device and inserted into the tumor tissue containing the plasmid DNA. Eight rectangular electric pulses of 99 μ sec with electric field strength of 1000 V/cm (applied tension of 9 volts) are delivered to the tumor tissue with 1 second interval through each pair of opposite electrodes. The procedure is repeated at four spaced tumor sites. Ganciclovir is administered to a patient at therapeutical doses, as in the second example, for the intracellular conversion of the nontoxic drug into the toxic compound by thymidine kinase expressed in the electroporated tumor cells with cytotoxic effect on these cells, and the cells surrounding them.

According to the present invention, the treatment of inherited beta-glucocerebrosidase deficiency (Gaucher's disease) is achieved by electroporation of normal liver tissue with plasmid

DNA carrying the gene of beta-glucocerebrosidase under the control of CMV promoter as described in Example 3.

In another embodiment of the present invention, the treatment of tumors of gastrointestinal origin including tumors of the stomach, pancreatic tumors of ductal phenotype
5 and tumors of duodenum is achieved through the noninvasive oral access to the tumor site using an endoscopic device as described above and electroporation procedure described in Example 3.

It should be noted that either DNA or RNA such as genes which cause living cells to express protein materials which are sensitive to specific drugs are preferred for use in treating tumors in accordance with this invention. Thus, a gene encoding the herpes virus thymidine
10 kinase can be injected into cells at a dosage rate of 10 mg/kilogram and preferably around 0.125 mg/kilogram, as set forth above, using the electroporation parameter set forth above to have the cells produce the protein thymidine kinase. When treated with ordinary dosages of ganciclovir, the treated cells which were treated by electroporation in vivo undergo a reaction between the ganciclovir and thymidine kinase to form a toxic substance which kills the transformed cells and
15 cells surrounding them.

In this case, ganciclovir which can be used can be Cytovene, a ganciclovir sodium sterile powder produced by Syntex Laboratories of Palo Alto, California. Dosages of about 1.25 to about 5mg per kilogram of body weight administered intravenously at a constant rate over one hour, every 12 hours, for fourteen to twenty-one days, can be used to treat tumors so as to kill
20 some or all of such tumors.

CLAIMS

1. A method of treating living mammalian cells in vivo comprising selecting living cells in vivo to be treated,
introducing foreign nucleic acid by electroporation into cells in vivo to cause
5 suppression or expression of at least one protein in vivo.
2. A method in accordance with the method of claim 1 wherein said cells are cancer cells.
- 10 3. A method in accordance with the method of claim 2 wherein said nucleic acid is a gene.
4. A method in accordance with claim 3 wherein said gene encodes for a protein which, when expressed, makes said cells sensitive to destruction when treated in vivo with a drug
15 nontoxic to normal body cells.
5. A method in accordance with the method of claim 4 wherein said gene is a gene encoding herpes virus thymidine kinase and said drug is ganciclovir.
- 20 6. A method in accordance with the method of claim 5 wherein said drug is intravenously administered.
7. A method in accordance with claim 1 wherein said foreign nucleic acid is selected from the class consisting of genes, antisense oligonucleotides, DNA, RNA.
- 25 8. A method in accordance with the method of claim 1 wherein said living cells are selected from the group consisting of organs, solid tumors, and tissues.
9. A method in accordance with the method of claim 8, wherein said living cells are
30 pancreatic tumor cells.

10. A method in accordance with claim 2 and further comprising said cells comprising a solid tumor,
and said electroporation being carried out on said tumor internally.

5 11. A method in accordance with claim 10 and further comprising the step of introducing electroporation electrodes to said tumor by the use of an internal cannula.

12. A method in accordance with claim 11 wherein said cannula is an endoscope carrying viewing optics.

10

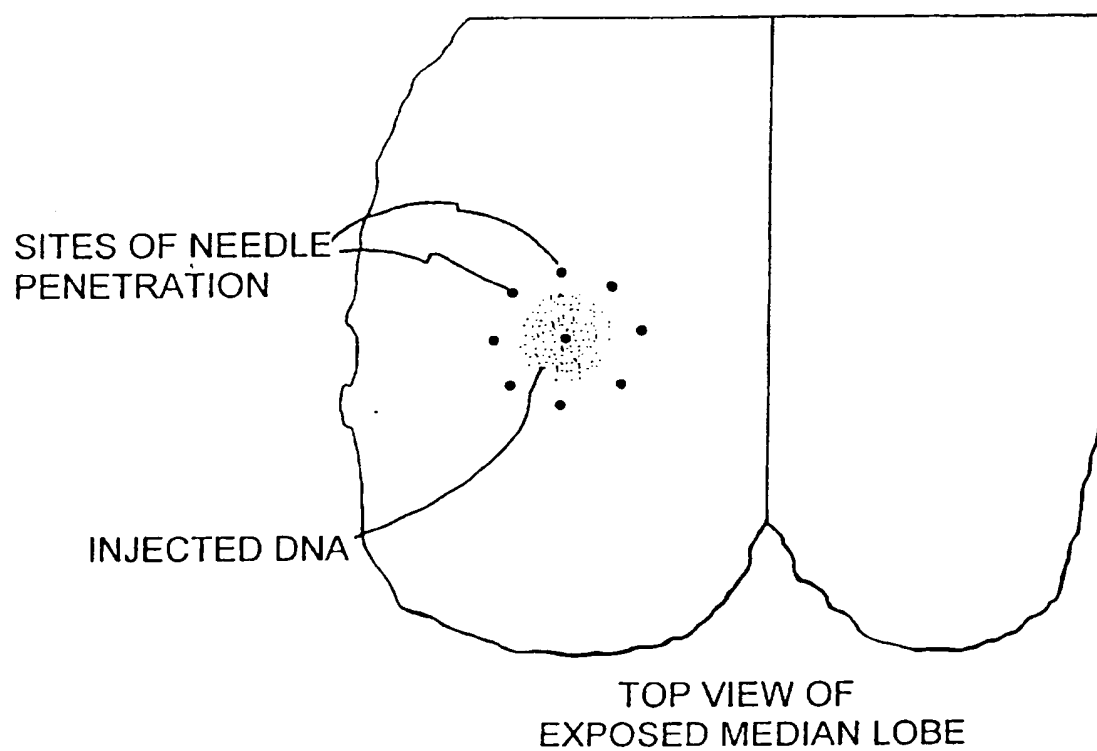
13. A treated living cell in vivo treated by the method of claim 2.

14. A treated living cell in vivo treated by the method of claim 4.

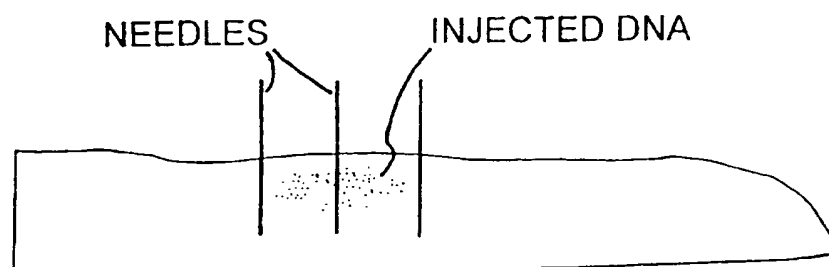
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15 15. A method of treating living mammalian cells in vivo, comprising:
selecting living cells to be treated in vivo,
introducing genetic material into said selected cells in vivo by electroporation to alter a genetic characteristic of said cells.

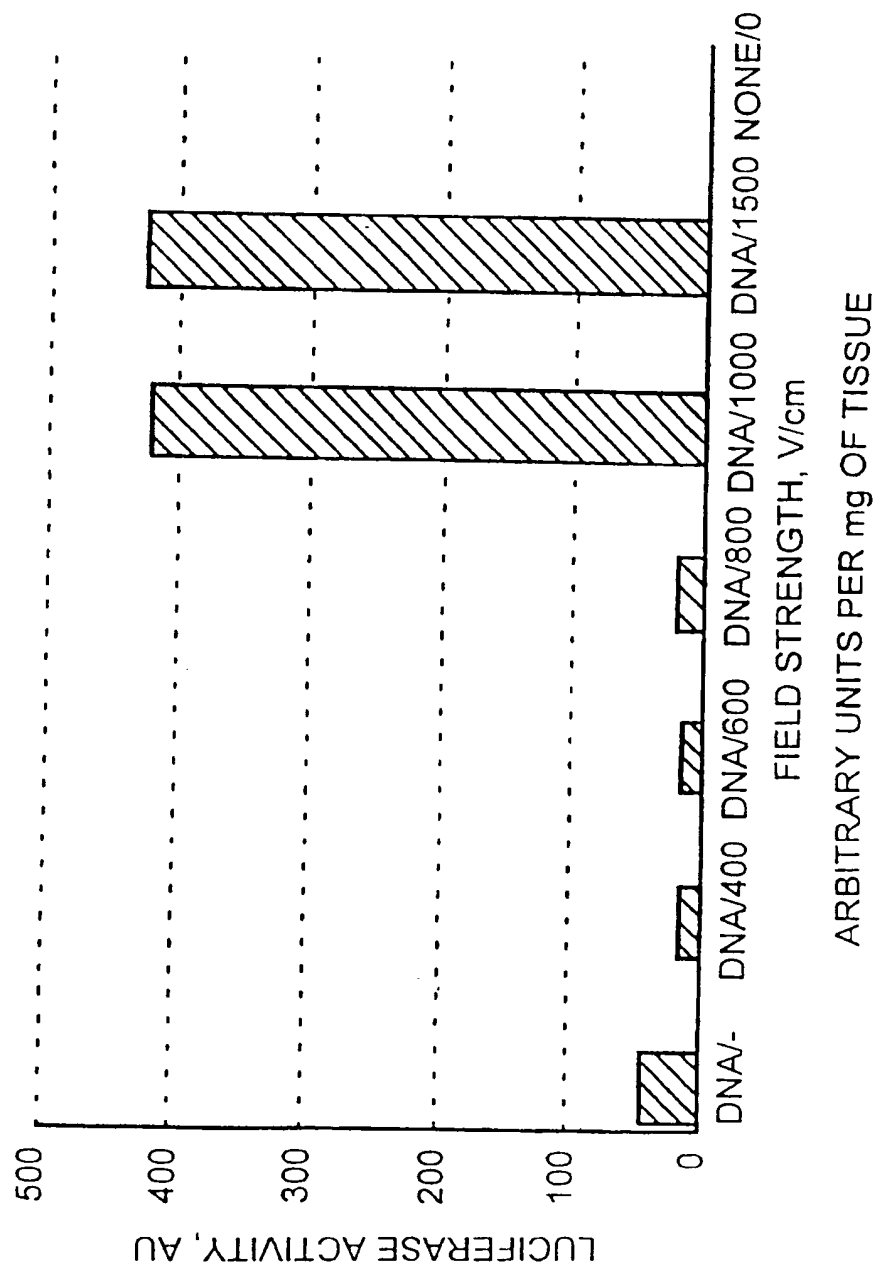
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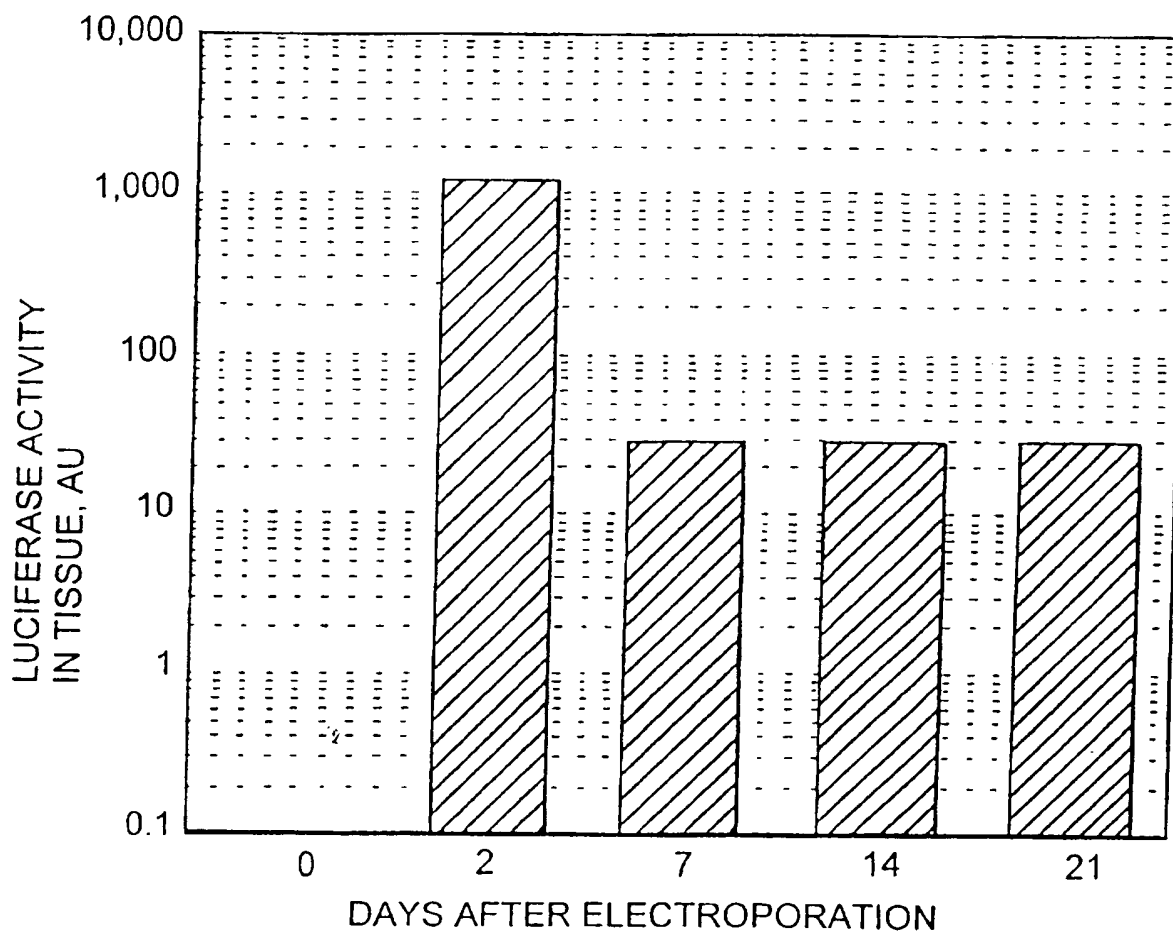
ELECTRODE PLACEMENT

Fig. 1CROSS-SECTIONAL VIEW OF
EXPOSED MEDIAN LOBE**Fig. 2**

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**Fig.3**

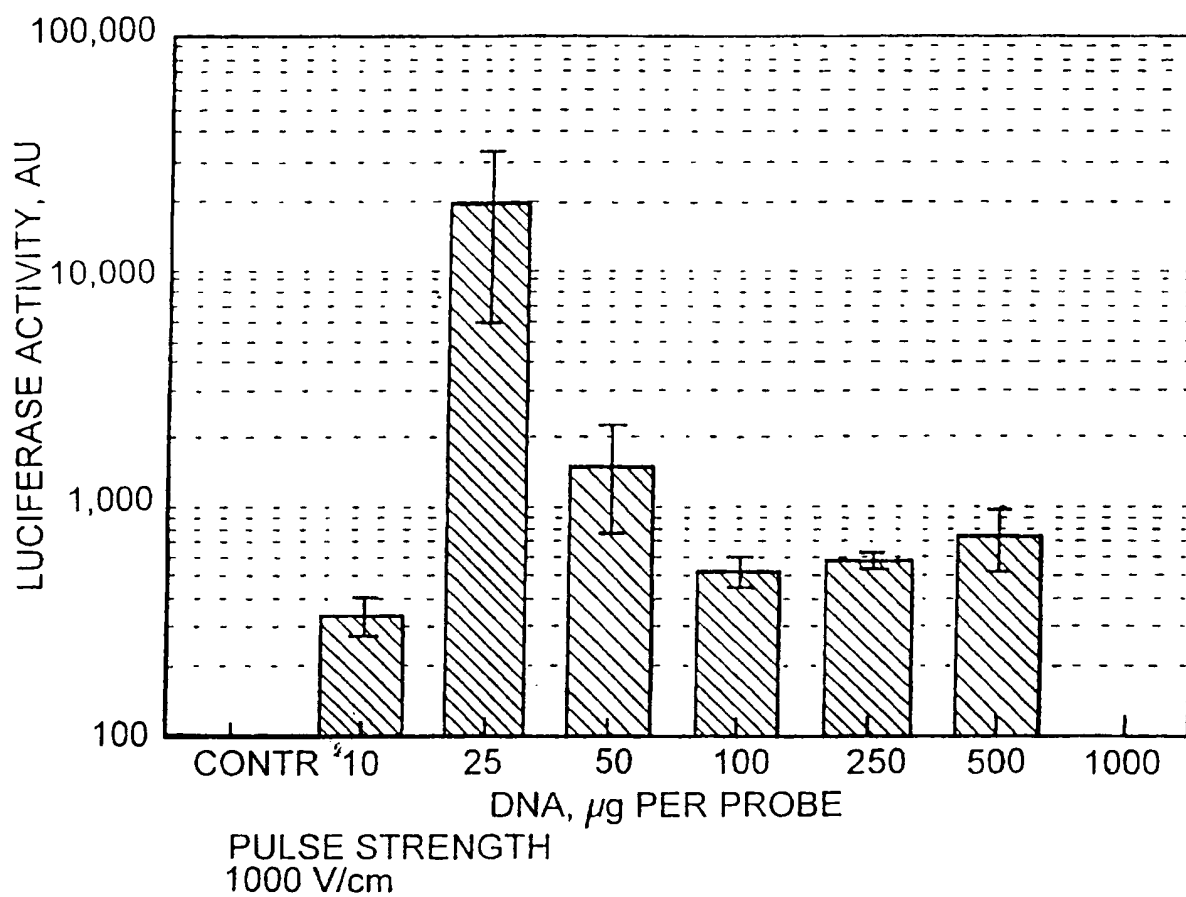
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TRANSIENT AND PERMANENT LUCIFERASE
EXPRESSION AFTER ELECTROPORATION

Fig. 4

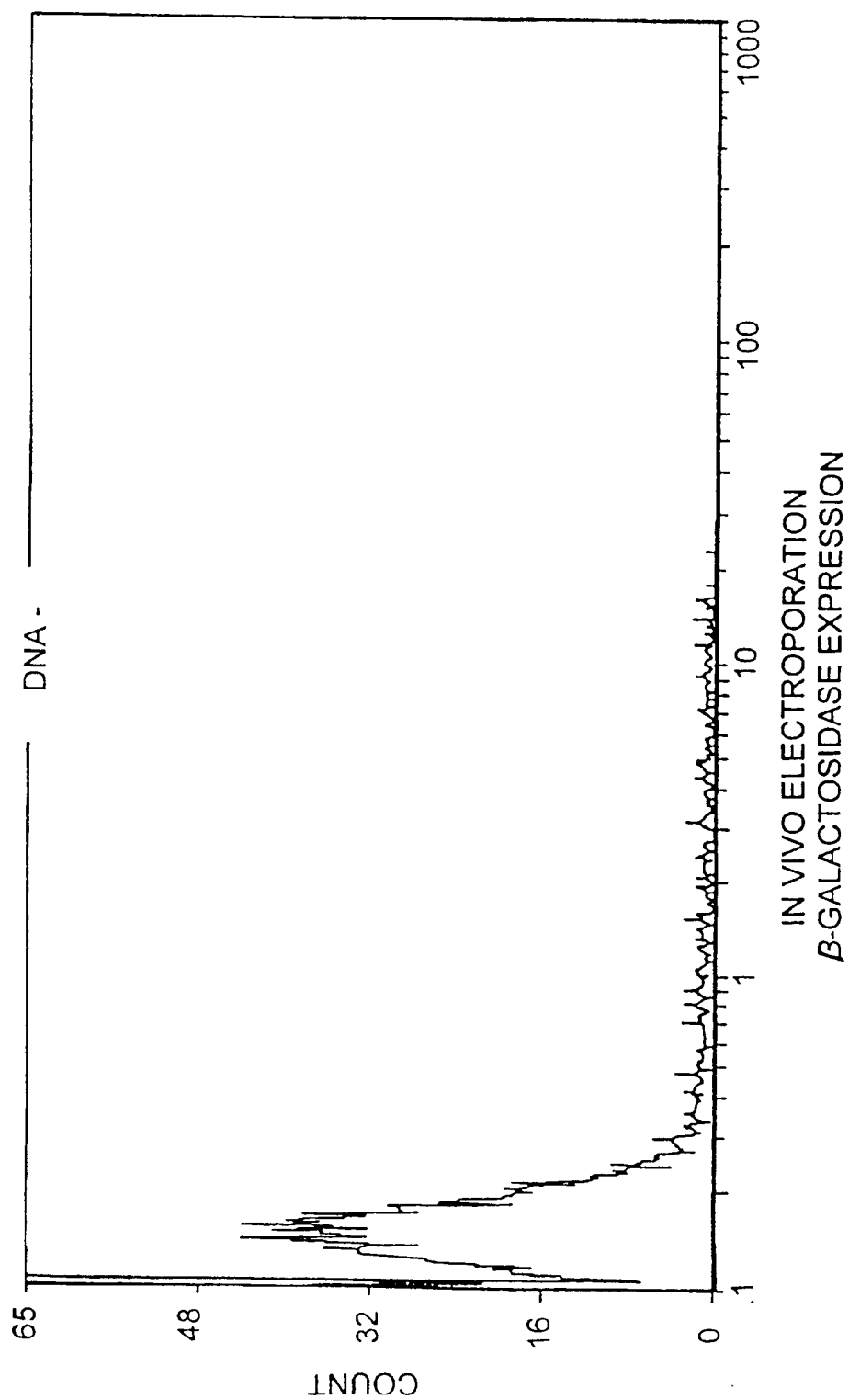
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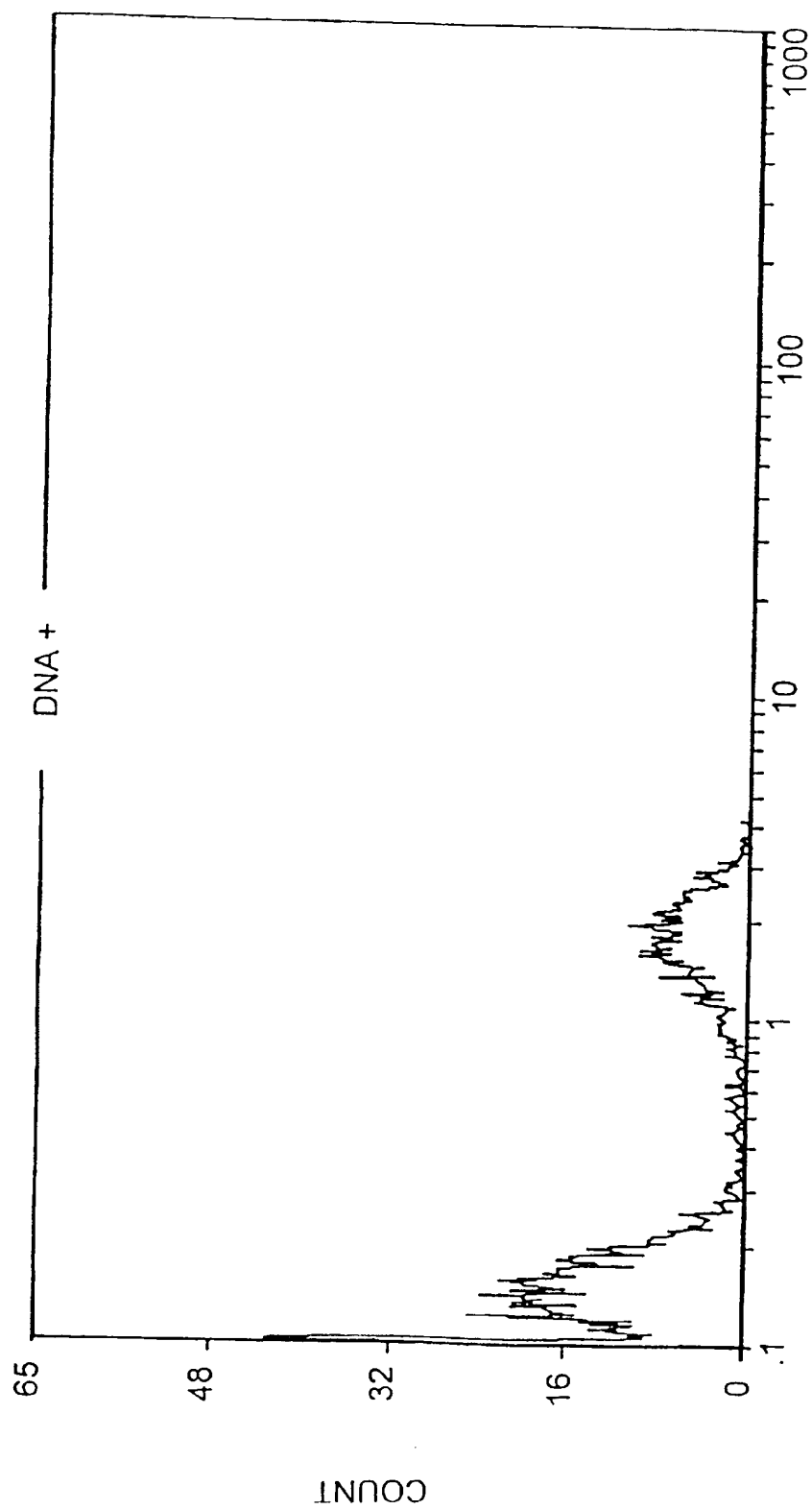
DNA DOSE-RESPONSE CURVE
RAT LIVER ELECTROPORATION IN VIVO

Fig. 5

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**Fig. 6**

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**Fig. 7**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13591

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 48/00; C12N 15/85

US CL : 514/44, 435/240.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/44; 435/240.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P ----- Y, P	NISHI et al. High-Efficiency in Vivo Gene Transfer Using Intraarterial Plasmid DNA Injection following in Vivo Electroporation. Cancer Research. 01 March 1996, Vol. 56, pages 1050-1055, see entire document.	1-3, 7-8, 10, 13, 15 ----- 4-6, 9, 11-12, 14
X, P ----- Y, P	GIORDANOY et al. In Vivo Gene Delivery to the Rabbit Carotid by Electroporation. Journal of the American College of Cardiology. February 1996, Vol.27, No.2, page 289A, abstract No. 780-4, see entire document.	1, 3, 7-8, 15 ----- 2, 4-6, 9-14
Y	SUKHAREV et al. Electrically-Induced DNA Transfer Into Cells. Electrotransfection In Vivo. Gene Therapeutics: Methods and Applications of Direct Gene Transfer. 1994, pages 210-232, see entire document.	1-15

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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Date of the actual completion of the international search 03 OCTOBER 1996	Date of mailing of the international search report 07 NOV 1996
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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LYERLY et al. Gene Delivery Systems in Surgery. Arch. Surg. November 1993, Vol. 128, pages 1197-1206, see entire document.	1-15